User manual

Check-Direct CPE

Real time PCR kit for the detection of carbapenemase-producing *Enterobacteriaceae*

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For use on: ABI 7500
Light Cycler®480 I&II
CFX96™
Rotor-Gene Q

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Intended use

Check-Direct CPE is a qualitative in vitro diagnostic test for the rapid detection of carbapenemase genes directly from perianal and rectal swabs. Check-Direct CPE detects the presence of the carbapenemase genes KPC, NDM, VIM, and OXA-48, presently the primary cause of carbapenemase production in *Enterobacteriaceae*. The assay is performed on an automated real-time PCR instrument using perianal/rectal swabs from individuals at risk of colonization with carbapenemase-producing *Enterobacteriaceae*. Check-Direct CPE can be used as an aid to identify, prevent and control carbapenemase-producing *Enterobacteriaceae* that colonize patients in healthcare settings. Check-Direct CPE is not intended to diagnose infections with carbapenemase-producing *Enterobacteriaceae* nor to guide or monitor treatment for these infections. Parallel cultures are necessary to recover organisms for epidemiological typing, susceptibility testing and for further confirmatory identification.

Introduction

The worldwide emergence and dissemination of carbapenem resistance among *Enterobacteriaceae* is a serious threat to public health. These organisms are associated with high mortality rates and have the potential to spread widely. The most common cause of carbapenem resistance in *Enterobacteriaceae* is the expression of carbapenemases, *i.e.* Carbapenemase-Producing *Enterobacteriaceae* or CPE. CPE have elevated or complete resistance to carbapenems and most other *β*-lactam antibiotics. Presently, the vast majority of CPE are associated with the presence of one of the following plasmid-encoded carbapenemases: KPC (*Klebsiella pneumoniae* carbapenemase), VIM (Verona integron–encoded metallo-β-lactamase), NDM (New Delhi metallo-β-lactamase) or OXA-48 (Oxacillinase-48). Moreover, CPE often have other non–*β*-lactam resistance determinants resulting in multidrug- and pandrug-resistant isolates.

Patients usually carry CPE by colonization of the colon. Therefore, rectal swabs provide a proper specimen to assess carriage of CPE, and perianal swabs may be used as a non-invasive alternative. Check-Direct CPE is a rapid real-time PCR test for the detection and discrimination of KPC, NDM/VIM, and OXA-48 in rectal and perianal specimens.

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Principle of the method

Check-Direct CPE assay is based on specific recognition and amplification of target sequences by PCR, and the simultaneous detection of the accumulation of PCR amplification products by fluorescent DNA probes. A control DNA molecule, the internal control, is added to the clinical specimen prior to DNA extraction to monitor that DNA extraction and PCR amplification were successful. Five molecular beacon probes, labeled with 4 different dyes are used to detect the various carbapenemases and the control DNA. Check-Direct CPE discriminates between KPC, NDM/VIM, and OXA-48. For each of the 4 carbapenemase genes, KPC, OXA-48, NDM and VIM, many gene variants exist. PCR primers and fluorescent probes of Check-Direct CPE are selected to target homologous gene segments of these carbapenemase genes, and in this way gene variants are reliably detected.

Kit contents (for 48 reactions)

Components (Mat. No.)	Description	Storage conditions
CPE PCR Mastermix (9-0080)	1 transparent tube and cap 630µl	+ 4°C
CPE solution (9-0071)	1 brown tube (purple inlay ●) 140 µl	- 20°C, **store in the dark**
Internal control (9-0077)	1 tube (red inlay ●) 300 µl	
Negative control (9-0070)	1 tube (white inlay ○) 100 µl	
KPC positive control (9-0073)	1 tube (green inlay ●) 100 µl	
NDM positive control (9-0075)	1 tube (gold inlay ●) 100 µl	
VIM positive control (9-0074)	1 tube (yellow inlay ●) 100 µl	
OXA-48 positive control (9-0076)	1 tube (orange inlay ●) 100 µl	
Manual (9-0078)	Leaflet – download from website	Not critical

Storage, handling, and stability

Check-Direct CPE reagents are shipped cooled. The CPE PCR Mastermix should be stored at +4°C upon receipt. All other reagents should be stored at -20°C upon receipt. Please visually inspect the box upon initial opening to ensure that its contents are intact. Check-Direct CPE solution should not be exposed to more than 12 freeze-thaw cycles. Please contact the Check-Points office at *support@check-points.com* if you have any further questions. Store kit reagents at indicated temperature until expiration date.

Materials required but not supplied with the kit

Supplies	Equipment
• NucliSENS® easyMAG® Extraction kit (bioMérieux, France) • Swabs for rectal and perianal specimen collection and transport (example: Sigma-transwabs (Medical Wire & Equipment, UK) or Eswab™(Copan Diagnostics, US) in Amies transport media). • Disposable laboratory (powder-free) gloves • Lab coat • Pipettes & disposable (filter-) tips for volumes of 1 to 1000 µl • 1.5 ml tubes (“Eppendorf tubes”) • 96-well PCR clear plate for use on ABI7500 • 96-well PCR white plate for use on CFX96™ and LightCycler®480 • PCR plate seal • PCR strip tubes and caps 0.1ml (QIAGEN,US) • For use on the LightCycler®480: the 4-Color Compensation Set (Ref: 18-0070, Check-Points Health B.V., NL)	• NucliSENS® easyMAG® Extraction platform (bioMérieux, France) • Real-time PCR instruments: ABI7500 (Applied Biosystems, US) LightCycler®480 I&II (Roche, CH) CFX96™ (Bio-Rad, US); Rotor-gene Q 5plex (QIAGEN, US) • Rotor-disc 72x0.1ml wells, locking Ring 72-Well Rotor, and loading block 72x0.1ml tubes (QIAGEN, US) • Vortex mixer • mini centrifuge • Plate centrifuge

Good laboratory practices

Recommendations for best results

- The test must be performed by adequately trained personnel.
- Do not use reagents after their expiration date.
- Before use, thaw frozen reagents completely at room temperature and vortex briefly to obtain a homogeneous solution. After vortexing briefly, spin down the solution to avoid contamination when opening the cap.
- Follow recommendations for storage, handling and freeze-thaw cycles to preserve the quality of the kit’s reagents.
- Protect reagents from light to avoid photo-bleaching of the dyes.
- Periodically, verify the accuracy and precision of pipettes, as well as correct functioning and calibration of the instruments.

Prevention of contaminations

Use separate rooms: a pre-PCR room and a post-PCR room.

- DNA extraction and preparation of the amplification reactions are carried out in the pre-PCR room.
- Incubation in the real-time PCR thermocycler is carried out in the post-PCR room.
- Never transfer items from the post-PCR room to the pre-PCR room.

To keep laboratory free of PCR product contamination:

- Use pipettes with hydrophobic filter tips.
- Make sure to always use a new pipette tip when adding solutions, test samples, and controls to wells of a 96-well plate.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean disposable gloves and clean lab coats for the different steps of the test.
- Change gloves whenever you suspect that they are contaminated.
- Keep the tubes of all kit components and samples closed as much as possible.
- Clean the lab benches and all equipment regularly with a 0.5% sodium hypochlorite solution.

Users are strongly advised to read the full protocol before starting the test

Specimen collection and DNA extraction

1. Human specimen collection

In order to obtain adequate specimen, the procedure for specimen collection must be followed carefully with adequate swab material (see section *Materials required but not supplied with the kit)*.

1. Collect perianal/rectal specimen according to local guidelines and swabs' manufacturer recommendations.
2. Place swabs in their containers, containing 1 ml of liquid transport medium.
3. Label the containers.
4. Refer to the swab manufacturer instruction for storage, handling, and stability.

2. DNA extraction from perianal/rectal swabs with NucliSENS® easyMAG®

Important points before starting: DNA extraction is carried out in the pre-PCR room.

Procedure:

1. Check-Direct CPE has been validated with the NucliSENS® easyMAG® automated DNA extraction procedure for perianal/rectal swabs in transport medium. Follow the protocol Generic 2.0.1 from the manufacturer for perianal/rectal swabs.
2. Use 200 µl of perianal/rectal swab fluid from each specimen and add 5 µl of the internal control solution (IC solution, ●) to each well of the easyMAG® cartridge. Start the DNA extraction using the Generic extraction protocol.
3. DNA is eluted in 70 µl elution buffer.
4. DNA extracts can be stored at +4°C for up to 6 months, and at -20°C for a longer period of storage.
5. Use the DNA solution directly and continue with the real-time PCR assay or store as specified until use.

3. Positive and negative control preparation

To validate the run, perform positive and negative control reactions for each Check-Direct CPE PCR run. The positive and negative controls are supplied with the kit.

- **Positive control(s)**

  Test the relevant positive control(s) provided in the kit. Positive controls contains the internal control.

- **Negative control(s)**

  Use the negative control (○) provided in the kit as a sample to validate the run. The negative control contains the internal control. We also recommend performing a DNA extraction as specified earlier, with the internal control solution using a sample known to be negative for the test in use (*i.e.*, carbapenemase negative sample, or elution buffer).

Real-time PCR assay

Important points before starting:

- Preparation of the real-time PCR reactions is carried out in the pre-PCR room.
- Specific parameters and materials should be used for each type of real-time PCR instrument use to perform the test.
- Targets are detected in the FAM, VIC, and TexasRed channels. The internal control is detected in the Cy5 channel.

1. Real-time PCR reaction setup

1. Calculate the number of reactions. Thaw reagents, mix well, spin down and keep on ice.
2. Prepare the real-time PCR (qPCR) reaction mix as described in Table 1. Multiply the CPE solution and the CPE PCR Mastermix by the right number of samples and include 10% surplus to ensure that you have enough qPCR reaction mix for all the calculated reactions.
3. Pipette 15 µl of qPCR reaction mix to the wells of the 96-well plate.
4. Pipette 10 µl of each test sample to the wells of the 96-well plate previously filled with 15 µl of qPCR Mastermix (see Table 2).
5. Pipette 10 µl of positive control (solution(s) KPC (●), NDM (●), VIM (●) and/or OXA-48 (●)) to the well of the 96-well plate previously filled with 15 µl of qPCR reaction mix (Table 2).
6. Pipette 10 µl of negative control solution (○) to the well of the 96-well plate previously filled with 15 µl of qPCR reaction mix (Table 2).
7. Seal the plate, or close the tubes. Mix the plate by tapping it on the bench and spin down briefly.
8. Transfer the plate to the post-PCR room.
9. **Without delay**, place the plate into the real-time PCR instrument and start the run with the parameters corresponding to the real-time PCR instrument in use (see next points). When the run is completed, discard the plate according to local regulations.

10. Following the manufacturer's instructions for each real-time PCR instruments, start and set up the run using the parameters specified in Table 3 and 4, and in the text below.

2. For use on the ABI 7500

- Run mode: Standard 7500
- Reaction volume: 25 µl
- ROX™ passive reference dye: Included in the qPCR Buffer
- TaqMan® Reagents
- Experiment: Quantitation – Standard Curve
- Standard ramp speed
- Targets: Reporter Dyes and Quencher setup, see Table 3.

3. For use on the LC®480 I&II

To use the Check-Direct CPE real-time PCR kit on the LightCycler®480 system I&II, a 4-color compensation object is required and generated by using the 4-Color Compensation Set supplied by Check-Points Health B.V. (Ref: 18-0070).

- Detection format LC®480 I: Multi Color Hydrolysis Probe
- Detection format LC®480 II: 4 color FAM VIC/HEX RED610 CY5
- Filter Combination Selection: see Table 3.
- Reaction Volume: 25 µl
- Analysis mode: Quantification for the amplification step
- Integration time mode: Dynamic
- Color compensation object or file required

4. For use on the CFX96™

- Sample Volume : 25 µl
- Temperature Control Mode : Calculated
- Scan Mode: All channel
- Plate type: BR white
- Plate Setup: View/Edit Plate and Select Fluorophore as described in Table 3

5. For use on the Rotor-Gene Q

- Reaction Volume : 25 µl
- 72-well rotor selected
- Gain settings: Green 6 / Yellow 4 / Orange 3 /Red 8.

Table 1: *qPCR reaction mix setup.*

Component	Volume per reaction
CPE PCR Mastermix	12,5 µl
CPE Solution (●)	2,5 µl
Total volume	15 µl

Table 2: *Real-time PCR sample setup.*

Reaction Type	Component	Volume per reaction
Test sample	Sample DNA	10 µl
Positive control	KPC (●), NDM (●), VIM (●), OXA-48 (●)	10 µl
Negative control	Negative sample or negative control provided (○)	10 µl

Table 3: *Real-time PCR setup.*

Target	ABI 7500 Detector	LC®480 I Detector	LC®480 II Detector	CFX96™ Detector	Rotor-Gene Q Detector	Reaction Volume Per Well
KPC	FAM	FAM (483-533)	FAM (465-510)	FAM	Green (510±5)	25 µl
NDM, VIM	VIC	VIC/HEX/Yellow555 (523-568)	VIC/HEX (533-580)	VIC	Yellow (557±5)	
OXA-48	TexasRed (TXR)	Red610 (558-610)	Red610 (533-610)	TexasRed (TXR)	Orange (610±5)	
Internal Control (IC)	Cy5	Cy5 (615-670)	Cy5 (618-660)	Cy5	Red (660±10)	

Table 4: *Real-time protocol parameters.*

Step	Temperature	Time	Cycles	Data Collection	Ramp Rate Mode	
					ABI 7500 /CFX96™/ Rotor-Gene Q	LC®480 I&II
1:	50°C	2 min	1	OFF	standard	4.4°C/s
2:	95°C	10 min*	1	OFF	standard	4.4°C/s
3: 4:	95°C 60°C	15 sec 60 sec	45	Plate read Optics on	standard	4.4°C/s 2.2°C/s

*3 or 10 min can be used for denaturation time depending on other assays to be run simultaneously with the the Check-Direct CPE test.

Data analysis

Important points before starting:

- For a detailed description on how to operate real-time PCR instruments and how to analyze data, please refer to the real-time PCR instrument instruction manual.
- Always visually inspect the amplification plot for each sample tested versus C_T values obtained with the software.

1. For use on the ABI 7500

1. Analyze data using ROX as a passive reference.
2. In the **Options** setting, for each **Target** (see Table 3), deselect the box for auto threshold and auto baseline as shown in Figure 1 (red circle) and select **Reanalyze.**
3. Set the threshold manually in the **Analysis settings** window for each detector channel using the threshold values recommended in Table 5.
4. Check amplification plots versus C_T values calculated by the software for each target.

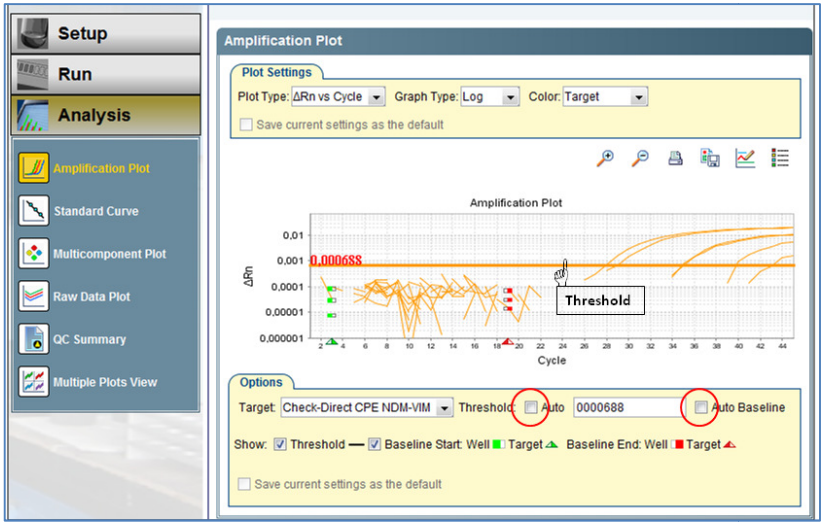

Figure 1: Screen shot of a typical analytical window on the ABI7500 with ABI7500 software v2.0.6
Amplification plot of positive and negative samples for the Check-Direct CPE test (logarithmic scale); Red circle: deselect the box of auto Threshold/Auto baseline.

2. For use on the LC®480 I&II

1. Select **Abs Quant/2nd Derivative Max** analysis (Figure 2).
2. Analyze the data using the **color compensation object** application-specific to the Check-Direct CPE assay previously created. Select **Color Comp (On)** – **In database** select the CC object (Refer to Check-Points User Manual for the 4-Color Compensation set, Ref: 18-0070).
3. Select the **High Sensitivity** option and **Calculate** C_p values for each filter combination.
4. For each filter combination, always check amplification plot versus C_p values.
5. In the results table, check C_p values for each targets versus the *Status* given by the software (red= positive; green= negative; blue= call uncertain). Validate the *Status* of the sample using the software by visually inspecting amplification curves (Figure 2).

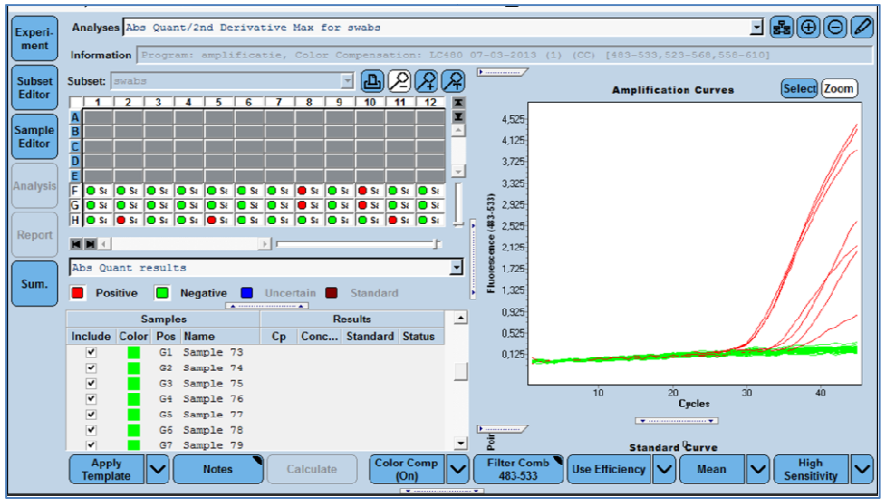

Figure 2: Screen shot of a typical analytical window on the LC®480 system I with the LC®480 software v1.5
Amplification plot of positive and negative samples for the Check-Direct CPE test (linear scale); threshold is automatically calculated by the software (Table 5).

Table 5: *Recommended threshold settings.*

Detector	Target	ABI 7500	LC®480 I&II	CFX™96	Rotor-Gene Q
FAM - green	KPC	0.002	Auto-calculated	60-150	0.02
VIC - yellow	NDM- VIM	0.001	Auto-calculated	200-250	0.015
TXR - orange	OXA-48	0.006	Auto-calculated	400-800	0.03
Cy5 - red	IC	0.003	Auto-calculated	40-100	0.06

3. For use on the CFX96™

1. Open the *Data file* for *Data Analysis*. In the **Analysis Settings** use the following parameters:
   - Analysis Mode : Fluorophore
   - Baseline Setting : Baseline Subtracted Curve Fit; Apply Fluorescent Drift Correction
   - C_q Determination : Single Threshold
   - Baseline Method : Auto Calculated
   - Cycle to Analyze: 10-45
   - Baseline Threshold: User defined (to be set per fluorophore)
2. Set the threshold manually for each detector channel using the threshold values recommended in Table 5
3. Check amplification plots (in the *Quantification* Tab) versus C_q values calculated by the software for all targets in the results table.

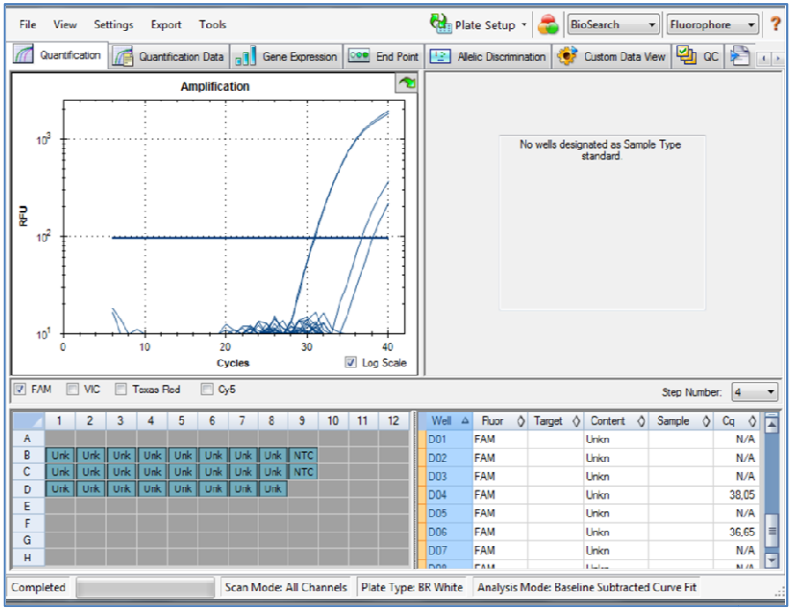

Figure 3: Screen shot of the typical analytical window on the CFX96™ with the CFX96™ software v2.0
Amplification plot of positive and negative samples for the Check-Direct CPE test (logarithmic scale); Adjust threshold manually using the threshold bar on the amplification plot; (Blue: FAM signal).

4. For use on the Rotor-Gene Q

1. Open the data file. Open the raw channel page for each detector. Select **Options** and **Crop start cycles**. In the pop-up window **Remove data before cycle**, enter "15" and select "**OK**".
2. Then, use the parameters indicated in Table 6 to analyze each detector channel.
3. Set the threshold manually for each detector channel using the value recommended in Table 5.
4. Check amplification plots versus Ct values calculated by the software for each target in the *Quant. Results* table.

Table 6: *Rotor-Gene Q analytical parameters*

Detector / TARGET	Dynamic Tube	Slope Correct	Outliners Removal	Eliminate Cycles
Green / KPC	ON	ON	10%	15
Yellow / NDM-VIM	ON	ON	6%	15
Orange / OXA-48	ON	ON	10%	15
Red / IC	ON	ON	15%	15

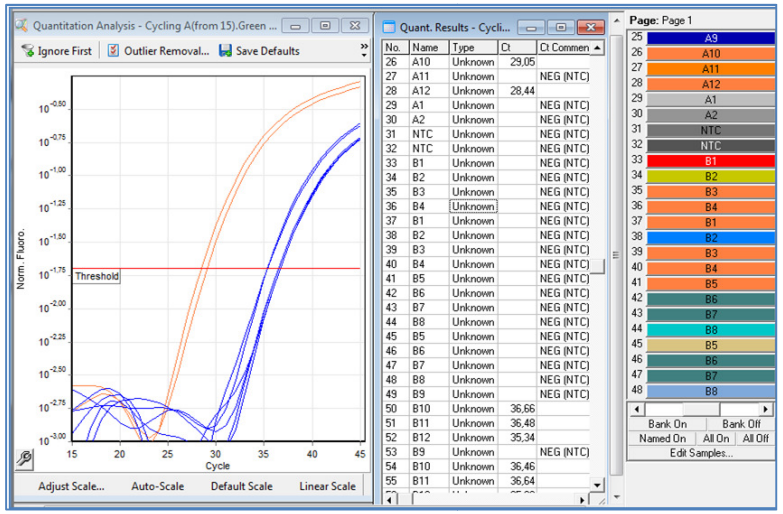

Figure 4: Screen shot of the typical analytical window with the Rotor-Gene Q software v2.1.0 (Build 9)
Amplification plot of positive and negative samples for the Check-Direct CPE test (logarithmic scale) in the Green channel.

Data interpretation

1. **Run validation: Table 6**
   Check the positive and negative control amplification curves. Valid run reports:
   - No instruments system failures during the run.
   - Negative control with a C_T value of 30 ±3 in the Cy 5 detector channel and no C_T value in the other detector channel.
   - Positive control C_T values as expected in Table 6. The exact C_T values of the positive controls vary depending on the qPCR instruments used and the threshold settings.
2. **Results interpretation: Table 7**
   If the run is valid, interpret results as positive, negative or invalid with the C_T values obtained for the samples with the guidelines summarized in Table 7:
   - Positive carbapenemase samples will show a C_T value in the FAM, VIC and/or Red(TXR) channel. Positive carbapenemase samples will also show a C_T value in the Cy5 channel if the target has not out competed the internal control (IC) for the resources during the reaction.
   - Negative carbapenemase samples will show no C_T value in the FAM, VIC, and Red(TXR) Channel. In the Cy5 Channel, a C_T value is expected at 30 ±3.
   - Samples with a FAM, VIC and/or Red(TXR) C_T undetermined or N/A, and with a Cy5 IC showing no C_T value or a C_T ≥33 mean that the sample is invalid, see FAQ and Troubleshooting 3 to7.

Table 6: *Criteria for a valid run with Check-Direct CPE test*.*

Sample Type / Expected C_T values	Instrument	KPC FAM - green	NDM VIC - yellow	VIM VIC - yellow	OXA-48 Red(TXR) – orange	IC Cy5 – red
Positive control	ABI7500, CFX™96, Rotor-Gene Q	25 ±3	29 ±3	26 ±3	24 ±3	30 ±3
Positive control	LC®480 I&II	28 ±3	30 ±3	27 ±3	26 ±3	30 ±3
Negative sample (extracted with IC)	FOR ALL	N/A	N/A	N/A	N/A	30 ±3

Table 7: *Data interpretation guidelines*.*

KPC, NDM/VIM, OXA-48 C_T values	IC C_T values	Interpretation
YES	YES or N/A	Positive sample
N/A	30 ±3	Negative sample
N/A	N/A or ≥33	Invalid

* If observed C_T values vary significantly from expected C_T values , see FAQ and Troubleshooting section

Frequently asked questions (FAQ) & Troubleshooting

1. **May other specimen preparation and DNA extraction methods be used with Check-Direct CPE?**
   Check-Direct CPE test has been optimized using specific swabs and transport medium in combination with the NucliSENS® easyMAG® extraction methods. Check-Points does not guarantee the performance of the test with methods other than those recommended in this manual.

2. **The real-time results show no C_T/C_P/C_q values or interpretation indicating that the sample is invalid.** Possible causes and troubleshooting:
   - The sample DNA was not added to the assay.
   - The sample DNA tested with Check-Direct CPE is negative and the internal control was not added prior to DNA extraction. Please repeat the DNA extraction.
   - The DNA extraction failed since the internal control was not detected. Please repeat the DNA extraction.
   - The sample DNA contains contaminants inhibiting the reactions. Please repeat the DNA extraction.
   - CPE Solution or CPE PCR Mastermix was not added to the assay. Please repeat the test.
   - Reagent solutions are degraded or may have expired.

3. **The real-time results show no C_T/C_P/C_q values for the positive control or interpretation indicating that sample is invalid.** Possible causes and troubleshooting:
   - The positive control solution was not added. Repeat the test.
   - CPE Solution or CPE PCR Mastermix was not added to the assay. Please repeat the test.
   - Reagent solutions are degraded or may have expired.

4. **Troubleshooting for invalid result.**
   Repeat the test using the same DNA extract. If invalid results persist, repeat the test by preparing a new DNA extract from the original specimen. Alternatively, repeat the test with a new DNA extraction from a newly collected specimen.

5. **Real-time results show very low fluorescent signals in all samples and detector channels, including the internal control signal.** Possible causes and troubleshooting:
   - The CPE solution containing the fluorescent probes and primers is degraded. Please check expiration date, the number of thaw/freezing cycles that the CPE solution tube has undergone, and if the kit was stored correctly.
   - The real-time PCR system may be responsible for these results. Please refer to instrument User's manual or contact your real-time PCR instrument local representative.

6. **C_T/C_P/C_q values troubleshooting.**
   - ***Samples with very low C_T values and no amplification curves.*** The manual threshold is too low. Samples showing a C_T value <15 and no amplification curve are considered negative. The observed C_T value is an artifact of the software analysis: the threshold crosses the background noise of the curve.
   - ***Invalid Run: C_T values expected for the controls in Table 6 do not match the C_T values observed* In the experiment.** Check that these differences are not due to the threshold being too high or too low. If changing the threshold does not improve the results, go to FAQ **3. to 6.**

7. **The real-time PCR instrument gives an error message.**
   Refer to the real-time PCR instrument user manual or contact the local technical support of the real-time PCR instrument company.

8. **I left Solutions (CPE, Internal control, negative, or positive control) out of the -20°C (-4˚F) storage.**
   These reagents must be stored at -20°C (-4°F) for proper performance of the test. The performance of the product cannot be fully guaranteed if these solutions were left out of -20°C (-4˚F) for more than 24 hours.

9. **Duplicate samples tested with Check-Direct CPE test did not yield identical results.**
   C_T (C_P/C_q) values of identical samples may vary between individual reactions. Large variations, > 2 C_T values, suggest pipetting errors or other differences between the duplicate samples.

10. **Data Analysis and Interpretation.**
    If you encounter difficulties with the data analysis and interpretation please contact Check-Points Technical Support at *support@check-points.com*.

Performance Characteristics

1. Analytical sensitivity

The analytical limit of detection (LOD) of Check-Direct CPE real-time PCR test was assessed for four carbapenemase genes associated with carbapenemase production in *Enterobacteriaceae*: KPC, NDM, VIM, and OXA-48.
No quantified genomic standards for these markers were available at the time, therefore the analytical sensitivity test was performed using plasmid having a 400 bp target sequence DNA fragment. Thus, the LOD of the Check-Direct CPE real-time PCR test was established using plasmid DNA directly in the PCR reaction mix. To determine analytical sensitivity, an end-point dilution was used until the assay could no longer detect the target in question in more than 5% of the replicates.

Results: See Table A.

Table A

Target	LOD (copies/PCR)
KPC	5
NDM	5
VIM	5
OXA-48	5

2. Specificity

2.1 *In silico* Specificity
The specificity of the Check-Direct CPE real-time diagnostic test is ensured by the selection of the correct primers and probes, as well as the selection of stringent reaction conditions. Primers and probes sequences were validated with *in silico* analysis. Primers and Probes sequences were designed to specifically identify the gene variants listed in Table B. Primers and Probes sequences were tested for potential homologies with all the gene sequences published by the international gene bank (GenBank®, NIH genetic sequence database) using sequence comparison analysis.

Results: No cross homology was found with other organisms for designed primers and probes

Table B

Gene	Variants
KPC	1 to 15
NDM	1 to 8
VIM	1 to 6, 8 to 37
OXA	48, 162, 163, 181, 204, 232, 244, 245.

2.2 Analytical Specificity

The experimental specificity of the Check-Direct CPE real-time diagnostic test was determined by testing the cross-reactivity with samples containing non-target organisms. 132 carbapenemase-negative strains were used to test the specificity of the Check-Direct CPE real-time test, see bacterial strains listed in Table C.

Results: All isolates tested negative with the Check-Direct CPE assay and the internal control was detected in all samples. The Check-Direct CPE test showed 100% specificity based on the reference strains tested.

Table C

Organisms	#	Organisms	#
Citrobacter freundii	5	*Enterococcus faecalis*	2
Campylobacter jejuni	2	*Klebsiella oxytoca*	1
Enterobacter aerogenes	1	*Klebsiella pneumoniae*	16
Enterococcus casseliflavus	1	*Pseudomonas aeruginosa*	2
Enterobacter cloacae	42	*Staphylococcus aureus*	2
Escherichia coli	51	*Salmonella thypimurium*	1
Pseudomonas mirabilis	3	*Stenotrophomonas maltophilia*	2
Serratia marcescens	1		

3. Analytical Reactivity

To evaluate the analytical reactivity, a retrospective study was performed with 93 bacterial strains of 26 different gram-negative species (Table D). The 93 bacterial strains tested with Check-Direct CPE were previously identified carbapenemase-positive with the micro-array diagnostics test Check-MDR CT103 (Check-Points Heatlth).

Results: All 93 bacterial strains were typed correctly for the targeted carbapenemase genes with the Check-Direct CPE test.

Table D

#	Check-MDR CT103	Check-Direct CPE
19	*KPC*	*KPC*
16	*NDM*	*NDM/VIM*
1	*NDM+OXA-48*	*NDM/VIM+OXA-48*
33	*VIM*	*NDM/VIM*
1	*VIM+OXA-48*	*NDM/VIM+OXA-48*
23	*OXA-48*	*OXA-48*

Key to symbols used

IVD	For *In Vitro* Diagnostic Use	CONTROL –	Negative control
REF	Catalog number	CONTROL KPC	KPC positive control
LOT	Batch code	CONTROL VIM	VIM positive control
IFU	IFU number	CONTROL NDM	NDM positive control
SOLUTION CPE	CPE solution	CONTROL OXA-48	OXA-48 positive control
INTERNAL CONTROL	Internal control	PCR Mastermix CPE	CPE PCR Mastermix
	Temperature limitation		Use before YYYY-MM
	Contains sufficient for < n > tests		Manufacturer
	Consult instructions for use		

Limitations

Check-Direct CPE is a DNA-based real-time PCR assay to detect the presence of the carbapenemase genes KPC, NDM, VIM, and OXA-48 in *Enterobacteriaceae*. The test detects the following carbapenemase gene variants: $bla_{VIM1\text{-}6;8\text{-}34}$;$bla_{OXA\text{-}48\text{-}162\text{-}163\text{-}181\text{-}232\text{-}244\text{-}245}$;$bla_{NDM1\text{-}8}$ and $bla_{KPC1\text{-}15}$. KPC, NDM, VIM, and OXA-48 represent the clinically most prevalent carbapenemases in *Enterobacteriaceae* in most parts of the world. However, other rare carbapenemases may also be responsible for carbapenemase production in *Enterobacteriaceae* and these are not detected by Check-Direct CPE. Carbapenem resistance is caused by carbapenemase production, but also by various other mechanisms. A negative result with Check-Direct CPE does not imply that the bacterium is not carbapenem resistant; it implies that the bacterium is not likely to carry any of the carbapenemase gene variants of KPC, NDM, VIM and OXA-48 detected by Check-Direct CPE. Therefore, the test result of Check-Direct CPE should never be used as guidance for therapy.

The quality of the input DNA is an important factor for obtaining reliable results with Check-Direct CPE. DNA must be extracted from perianal or rectal swabs in transport medium using the NucliSENS® easyMAG® DNA extraction system (bioMérieux, FR). Other DNA extraction systems have not been approved for use with Check-Direct CPE yet. The assay has been validated for both Sigma-transwabs (Medical Wire & Equipment, UK) and Eswab™(Copan Diagnostics, US) in Amies transport media. Other swab types are also expected to work well but this has not been validated yet.

The assay has been tested extensively with samples containing various gram-negative bacteria, such as *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Pseudomonas*, with excellent results. However, it may never be excluded that other Gram-negative bacteria or certain strains of the above species will yield poor results. Check-Direct CPE cannot and does not make any representation or warranty that it is capable of correctly detecting KPC, NDM, VIM, and OXA48 in all gram-negative species, subspecies or types or in all clinical sample sources. Results may need to be confirmed by additional methodologies in specific cases (*e.g.* for regulatory samples). Due to the high variability of bacterial genomes it is possible that certain subtypes might not be detected. The test reflects the state of knowledge of Check-Points Health B.V.

As with other diagnostic assays, the results of this test may only be interpreted in combination with additional laboratory and clinical data available to the responsible person. Use of this assay is limited to appropriately qualified personnel, well-trained in performing DNA-based molecular detection methods.

Technical assistance

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Despite the utmost care in the development and preparation of the protocol Check-Points cannot take any responsibility for errors, omissions and/or future changes herein.

Literature Citation: When describing a procedure for publication using this product, please refer to it as the *Check-Direct CPE*.

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